Beneficial Effects of Dietary Mineral Restriction in Dogs with Marked Reduction of Functional Renal Mass\textsuperscript{1,2}


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Abstract

Although studies in partially nephrectomized rats have identified a progressive nephropathy that is altered by dietary restriction of phosphorus intake, the response of dogs to similar perturbations has not been established. Functional renal mass was reduced by 15/16 in dogs to determine its long-term effects on renal function and to evaluate the effects of two levels of dietary mineral (calcium and phosphorus) intake (0.44% phosphorus/0.57% calcium versus 1.50% phosphorus/1.91% calcium). Following a 3-month stabilization period, dogs were fed either the lower mineral diet (group 1, \(N = 12\)) or the higher mineral diet (group 2, \(N = 12\)) for 24 months. Loss of renal function with the passage of time was observed in 10 of 12 dogs maintained on the higher mineral diet, with an average decrease in exogenous creatinine clearance of 11.1 \(\pm\) 6.3%/month, leading to a survival rate of 33% in this group. Restriction of dietary mineral intake slowed \((P < 0.05)\) the rate of decline of exogenous creatinine clearance in group 1 to 2.6 \(\pm\) 1.1%/month and improved 24-month survival to 75% \((P < 0.01)\). Deterioration of renal function was associated with renal calcium accumulation and histologic evidence of nephrocalcinosis, tubular atrophy and dilatation, and interstitial fibrosis. These events were more readily apparent in female than in male dogs. A role for glomerulosclerosis was not apparent, and neither glomerular pathology nor glomerular volume was related to the observed decrements in renal function.

Key Words: Renal failure, nephrocalcinosis, phosphorus toxicity, glomerulosclerosis

After partial nephrectomy (Nx),\textsuperscript{4} certain strains of rats develop a nephropathy characterized by glomerulosclerosis (1,2), tubulointerstitial disease (3–5), and progressive renal insufficiency. Although this progressive nephropathy has been well characterized in rats, it is unclear whether the same changes occur in other species (6). Human beings with renal disease frequently have a decline of glomerular filtration rate (GFR) with time (7), but it is difficult to separate the effect of clinical disease from a self-injurious property of the kidney. Evaluation of uninephrectomized human beings with no preexisting renal disease yields no evidence of progressive decline of GFR (8).

The dog has been used extensively as a model for the study of chronic renal failure, but only a few studies have directly addressed the issue of the progressive nature of renal disease in this species. No evidence of progressive decline in GFR was found in one 4-yr study of 3/4 Nx dogs on three diets varying in protein content (9,10) or in more severely Nx dogs fed three diets varying in protein and phosphorus content for 3 months (11). Another study identified progressive proteinuria in partially Nx dogs on a high-phosphorus, high-protein diet (12), but this pattern of urinary protein loss was not accompanied by a progressive decline of GFR. When 7/8 Nx dogs were studied for variable time periods of 15 to 39 months (13), 7 of 10 had no evidence of declining renal function. These studies clearly establish that the temporal course of renal function after partial Nx differs between dogs and rats. It remains to be determined

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\textsuperscript{4} Although not strictly correct, partial Nx here refers to the commonly employed procedure of partial transection of one kidney followed by contralateral Nx.

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whether a progressive decline in renal function occurs consistently, or at all, in partially Nx dogs.

Interest in the progressive nephropathy of rats has included studies on the role of dietary intake of phosphorus. It has been shown that reduction of dietary phosphorus delays or prevents progressive declines of renal function in partially Nx rats (3,14) and in rats with primary glomerular disease (15). The mechanism of protection with phosphorus restriction is unclear, although reduced nephrocalcinosis (3,14–18), reduced glomerulosclerosis (14), altered lipid metabolism (14), reduction of plasma parathyroid hormone (PTH) concentrations (19), or prevention of renal cellular hypermetabolism (20) have been implicated as protective effects.

At least three factors beyond total dietary phosphorus intake make important contributions to the renal toxicity of phosphorus in rats. First, a reduction of the dietary calcium:phosphorus ratio worsens nephrocalcinosis, an effect which is independent of total dietary phosphorus intake (21–23) and which has been implicated by some investigators (24) as the principal cause of nephrocalcinosis in studies employing calcium:phosphorus imbalanced diets. Second, nephrocalcinosis is more prevalent in female than male rats (21,22,25), an effect which may be estrogen linked (26). Finally, predisposition to renal calcium deposition appears to be genetically determined, with some inbred strains of rats highly susceptible and outbred strains apparently more resistant (23).

Whereas glomerulosclerosis appears to be uncommon in dogs with proteinuria (27), nephrocalcinosis and associated tubulointerstitial lesions are noted frequently in kidneys from dogs with naturally occurring chronic renal failure (28). To determine if development of renal lesions in a genetically heterogeneous population was related to dietary phosphorus intake, we evaluated the effects of balanced restriction of dietary mineral (phosphorus and calcium) on normal residual renal tissue in male and female mongrel dogs after partial Nx. A severe degree of renal mass reduction might be expected to expose a course of declining renal function in partially Nx dogs, if one exists. Consequently, we chose to study dogs with 15/16 Nx.

### METHODS

#### Experimental Animals

Experiments were performed on random source, young adult mongrel dogs of both sexes. Dogs were procured from the University of Georgia College of Veterinary Medicine Laboratory Animal Facility. Dogs were vaccinated against common viral pathogens and leptospirosis, treated for ectoparasites and endoparasites, and tested for *Dirofilaria immitis* microfilaria. They were given a commercial brand of dry dog food (24.5% protein by dry wt) and water *ad libitum*. After 21 days of quarantine, 29 clinically normal dogs were accepted for study on the basis of being negative for microfilaria and having a normal urinalysis and a negative urine culture for bacteria. All experimentation was conducted in accord with the NIH Guide for the Care and Use of Laboratory Animals.

Twenty-four dogs, subsequently described as groups 1 and 2, had renal mass reduced in a two-step procedure. Initially, dogs anesthetized with halothane had the left kidney exteriorized via a flank incision and approximately 7/8 of the kidney was infarcted by ligation of interlobar renal arteries and their branches. One week later, contralateral Nx was performed by using halothane anesthesia. A portion of the right kidney was preserved in 10% neutral buffered Formalin solution at the time of Nx, and the remaining portion was frozen for subsequent analysis for mineral content. This procedure, infarction of 7/8 of the left kidney followed by contralateral Nx, is subsequently referred to as 15/16 Nx.

Five dogs not subjected to surgical procedures served as controls (group 3) to determine the degree of reduction of renal function in groups 1 and 2.

#### Study Protocol

The study was divided into two stages. Stage I, of 3-months duration, occurred from the time of Nx until the initiation of the feeding trial. This stage allowed for compensatory renal growth and stabilization of renal function. Dogs were given water and diet 1 (Table 1) during this period. Plasma concentrations of creatinine, phosphate, calcium, triglycerides,

### TABLE 1. Composition of diets expressed on a dry matter basis

<table>
<thead>
<tr>
<th>% Nutrient</th>
<th>Protein</th>
<th>Fat</th>
<th>Carbohydrate</th>
<th>Fiber</th>
<th>Ash</th>
<th>Sodium</th>
<th>Potassium</th>
<th>Magnesium</th>
<th>Calcium</th>
<th>Phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet 1</td>
<td>16.7</td>
<td>11.9</td>
<td>62.0</td>
<td>1.82</td>
<td>3.16</td>
<td>0.33</td>
<td>0.48</td>
<td>0.13</td>
<td>0.57</td>
<td>0.44</td>
</tr>
<tr>
<td>Diet 2</td>
<td>17.0</td>
<td>10.8</td>
<td>60.3</td>
<td>1.78</td>
<td>7.64</td>
<td>0.35</td>
<td>0.48</td>
<td>0.15</td>
<td>1.91</td>
<td>1.50</td>
</tr>
</tbody>
</table>
and cholesterol, and urine concentrations of creatinine and phosphate (Gilford Instruments Laboratories, Inc., Oberlin, OH), plasma electrolytes (Beckman Instruments Inc., Irvine, CA), urine protein concentration (29), renal exogenous creatinine clearance (CCR), fractional excretion of phosphate (FEP), the ratio of urine protein concentration to urine creatinine concentration (Uprotein/Uprotein) as an estimate of proteinuria (30), and an oscillometric measure of mean arterial pressure (MAP) (31,32) were determined 2 and 3 months after renal mass reduction. Plasma immunoreactive parathyroid hormone concentration (PTH) was measured by an intact hormone radioimmunoassay (Nichols Institute, San Juan Capistrano, CA) that has been previously validated in the dog (33).

Stage II (feeding trial) was 24 months in duration. At the time of the initiation of stage II, the dogs were divided randomly into two groups of 12 dogs, with five female dogs in each group and with no difference in initial CCR, renal insulin clearance (Clin), hematocrit, Uprotein/Uprotein, MAP, or plasma concentrations of creatinine, phosphate, calcium, PTH, total CO2, triglycerides, cholesterol, or total protein (Table 2). During stage II, daily monitoring included measurement of food intake and observation of physical status and activity of individual dogs. Monthly measurements included determination of hematocrit and plasma concentrations of creatinine, urea nitrogen, phosphate, calcium, sodium, potassium, chloride, total CO2, and total protein. Every 4 months of stage II, plasma triglyceride and cholesterol concentrations, CCR, FEP, MAP, and Uprotein/Uprotein were determined in the partially Nx dogs. Plasma PTH concentration was determined at the initiation and at 2, 12, and 24 months of stage II. All determinations were repeated when dogs developed end-stage renal failure, as judged by the presence of marked elevations of plasma creatinine concentrations (>10 mg/dL), reduced Clin (<0.25 ml/min/kg), and reduced food intake. The Clin was measured at the initiation and termination of stage II for each dog. For analysis, terminal measurements were grouped with the next scheduled triennial measurement.

At 12 and 24 months of stage II, dogs were placed in metabolism cages to allow timed, complete collections of urine. Dogs were acclimated to the cages for 48 h before urine collections. A sample of urine was analyzed for protein and creatinine concentration, and a blood sample was obtained at the beginning and end of each collection period for measurement of serum creatinine and phosphate. Endogenous CCR, FEP, and 24-h urinary protein excretion were calculated. Reported results represent the average of two sequential 24-h urine collections at each time period.

Dogs were sacrificed by i.v. injection of sodium pentobarbital, either after renal functional measurements at the end of 24 months or when they devel-

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Body wt (kg)</th>
<th>Plasma Phosphate (mg/dl)</th>
<th>Plasma Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>13.0 ± 1.2</td>
<td>0.769 ± 0.046</td>
<td>3.22 ± 0.36</td>
</tr>
<tr>
<td>Group 2</td>
<td>15.0 ± 1.5</td>
<td>0.784 ± 0.047</td>
<td>3.88 ± 0.4</td>
</tr>
<tr>
<td>Group 3</td>
<td>14.0 ± 1.4</td>
<td>0.781 ± 0.036</td>
<td>3.77 ± 0.33</td>
</tr>
</tbody>
</table>

* Values are mean ± SE. All dogs were fed diet 1 (low mineral content) during stage I (3 months in duration). Values within the same group are significantly different (P < 0.05). ND: not determined. Mean PTH in a population of 50 intact dogs, 41 ± 3 ng/ml (P < 0.05 versus groups 1 and 2).
oped end-stage renal failure. Complete necropsies, with tissue collection, were performed on all dogs. The remnant kidney was weighed and cut in half along the sagittal plane. One half was preserved in 10% neutral buffered Formalin solution for light microscopic studies; the other half was frozen for subsequent tissue mineral analysis.

Five control dogs (group 3) were fed diet 1 during stage I. They served as controls to determine the degree of reduction of renal function in groups 1 and 2 at the end of stage I. They were not studied during stage II.

Diets

Analysis (Theracon Corporation, Topeka, KS, and Woodson-Tennent Laboratories Incorporated, Gainesville, GA) of the two experimental diets (Theracon Corp.) indicated they were similar in protein, fat, carbohydrate, and calorie contents but markedly different in total phosphorus and total calcium contents (Table 1). Total phosphorus and calcium contents of diet 1 were reduced by approximately 70%. The calcium:phosphorus ratio was 1.3:1.0 in both diets. Both diets were moderately low in protein content. Fatty acid content of diets 1 and 2, determined in triplicate by gas chromatography, indicated no difference in content of polyunsaturated fatty acids (33.7 ± 0.3 and 33.1 ± 0.2% of total fatty acid content, respectively), which were predominantly linoleic acid (92.7 ± 1.4 and 92.0 ± 0.5%, respectively).

For the feeding trial, dogs were housed individually in climate-controlled rooms. Dogs were fed a measured quantity of food calculated to provide their daily metabolic energy requirement of 132 kcal/kg^{0.75}/day (34).

Clearance Measurements

Renal clearance measurements were made after a 12- to 18-h fast. While restrained in slings to which they had been previously acclimated, the dogs were given water equal to 3% body wt (vol/wt) by gavage. A bolus injection of creatinine (40 mg of creatinine/kg body wt) was given i.v. and followed by a continuous infusion of 0.18 mg of creatinine/kg/min delivered in normal saline solution at an infusion rate of 2.0 mL/min. Urine was collected by an indwelling bladder catheter, and CCr was determined as the average of three consecutive clearance periods of at least 20 min each. This rate of creatinine infusion maintained plasma creatinine concentration at a constant level which was between 8 and 14 mg/dL. For simultaneous Cln determinations, a bolus injection of 0.12 μCi/kg of [14C]inulin (Amersham Corp., Arlington Heights, IL) was given i.v. and continuous administration (0.002 μCi/kg/min) was delivered in the 2-mL/min saline infusion. In all cases, the CCr differed from simultaneous Cln determinations by less than 5% (r = 0.986; N = 48) and the relationship between CCr and Cln was unaffected by diet, time, or gender of dog. Concentrations of creatinine and phosphate in urine and heparinized blood plasma were analyzed by using an automated device (Gilford Instruments Laboratories Inc.). Analysis of plasma and urine for [14C]inulin was performed by liquid scintillation (Beckman Instruments Inc.). Clearance values and FEP were calculated by standard formulae.

Morphologic Studies

The preserved renal tissues were dehydrated and embedded in paraffin. Two sections from both kidneys of each dog were stained with hematoxylin/eosin, periodic acid-Schiff's, Von Kossa, and Alizarin red stains. For morphologic studies, zones of renal cortex containing small glomeruli adjacent to areas of infarction were excluded from analysis. Without prior knowledge of group, two individuals (S.A. Brown and W.A. Crowell) examined the slides for the presence of glomerular, vascular, and tubulointerstitial lesions by using a qualitative scoring system (0, no change; 1, mild; 2, moderate; 3, severe change). Sections from each kidney were separately given an overall score for nephrocalcinosis, fibrosis, and tubular atrophy/dilation. For each kidney, as an index of the overall severity of tubulointerstitial lesions, the tubulointerstitial index was taken as the mean of the scores for nephrocalcinosis, fibrosis, and tubular atrophy/dilatation. A minimum of 25 glomeruli were viewed by each examiner, and each glomerulus was assigned an individual score from 0 to 3 to reflect the severity of mesangial matrix expansion and the degree of increased cellularity. An index equal to the mean of the scores for mesangial matrix expansion and for cellularity was assigned to each glomerulus. A mean glomerular index was then determined for each kidney by the averaging of the indices for all examined glomeruli. Cross-sectional areas of glomerular capillary tufts and proximal tubular epithelial profiles in Formalin-fixed tissue were measured with a planar morphometry image analysis system (Southern Micro Instruments Inc., Atlanta, GA). The morphometry system was calibrated with the aid of a micrometer reticle (Reichert Scientific Instruments, Buffalo, NY). Cross-sectional area was measured for 20 outer cortical and 20 juxtamedullary glomeruli, chosen randomly without knowledge of group. No correction was made for shrinkage occurring during the fixation process. Glomerular volume was calculated for each dog by the formula V = β/K (Area)^22, where β = 1.38, the shape coefficient for spheres, K = 1.1, the size distribution...
coefficient for glomerular profiles, and Area = the mean planar area of the 40 measured glomerular profiles (35).

Mineral Analysis

The sagittal half of the remnant kidney was trimmed to exclude nonviable renal tissue resulting from ischemia after vascular ligation. Outer cortex from both the normal kidney and the trimmed remnant of each dog was separated from the rest of the kidney by sharp dissection. Both the outer cortical and the inner cortical/medullary portions were weighed. Both parts of each kidney were separately homogenized in a tissue blender (Eberbach, Ann Arbor, MI). Duplicate weighed samples (approximately 1.0 g) of each part were ashed at 550°C for 15 h and subsequently reconstituted with 10 mL of a solution of 30% concentrated nitric acid and 10% concentrated hydrochloric acid in deionized water. Sample concentrations of calcium, magnesium, and phosphorus were determined by plasma mass spectrometry.

Statistical Analysis

Reported values are mean ± SE. Statistical analyses were performed with the aid of a commercial software package (Brainpower Incorporated, Calabasas, CA). Group comparisons of quantitative data were obtained by analysis of variance with repeated measures. Where a significant effect was identified, group means were compared by the Fisher's protected least significant difference test. For statistical analyses, the log10 transformation of renal calcium content was employed to adjust for skewness due to a few large values in each group. Two group comparisons of qualitative data (morphology) were made by the nonparametric Mann-Whitney U test. When three or more groups of qualitative data were compared, the Kruskal-Wallis H test was used as a global test to control per experiment error rate, and intergroup comparisons were made by the Mann-Whitney U test. To determine the rate of change of renal function over time, a linear regression model was derived for each animal, expressed as CCr = (slope × time) + intercept, using the serial values for CCr (expressed as percentage of baseline values) and the slope (% change per month) was taken as the rate of change of renal function for that animal. A P value of <0.05 was considered indicative of a statistically significant difference.

RESULTS

After surgical reduction of renal mass, all partially Nx dogs (N = 24) were maintained on diet 1 for 3 months to standardize any dietary effects on renal function and structure during stage I. In the 24 partially Nx dogs, mean CCr was 0.660 ± 0.37 mL/min/kg when measured 2 months after renal mass reduction and 0.747 ± 0.043 mL/min/kg at the time of initiation of stage II 1 month later (P < 0.001). This represented an increase (P < 0.05) in CCr of 13.2 ± 2.7% in the final month of stage I.

The values for CCr at the end of stage I, before the dietary trial, represented an average reduction of 77.1 ± 1.3% in the 24 partially Nx dogs (groups 1 and 2) when compared with that in control dogs (group 3) maintained on the same diet (Table 2). The degree of reduction of Cln was nearly identical (76.5%). There were significant elevations in FEP, plasma PTH, Uprot/UCr, MAP, and plasma concentrations of phosphate, calcium, triglycerides, and cholesterol in the partially Nx dogs (groups 1 and 2) compared with that in control dogs (group 3, P < 0.05) at the initiation of stage II.

During stage II, there were no differences between groups 1 and 2 in mean daily food intake (17.4 ± 0.4 versus 17.2 ± 0.3 g/kg; not significant [NS]), daily energy intake (64.4 ± 1.6 versus 62.1 ± 1.1 kcal/kg; NS), daily protein intake (2.74 ± 0.07 versus 2.83 ± 0.05 g/kg; NS), or body weight (Table 3) during the study. Mean daily phosphorus intake in group 2 (249.3 ± 4.5 mg/kg) exceeded the corresponding value for group 1 (71.4 ± 1.8 mg/kg; P < 0.0001).

As an index of daily protein and phosphate excretion, 24-h urine collections were obtained at 12 and 24 months of stage II. Mean values indicated a significantly lower daily phosphate excretion in group 1 at 12 months of 191.4 ± 23.4 mg/day compared with the mean value of 416.2 ± 43.0 mg/day in group 2 (P < 0.001). Similar values for mean daily phosphate excretion (203.1 ± 18.4 in group 1 versus 450.0 ± 53.7 mg/day in group 2; P < 0.001) were obtained at 24 months, confirming the differences in dietary availability of phosphorus. Daily protein excretion at 12 months averaged 478.5 ± 123.1 mg in group 1 and 535.6 ± 115.8 in group 2 (NS), and mean values were not different at 24 months (498.6 ± 116.9 mg of protein/day in group 1 and 464.0 ± 259.9 mg of protein/day in group 2). There were strong correlations between 24-h endogenous and short-term exogenous CCr measurements (r = 0.910; P < 0.001). FEP determined by 24-h collections and by short-term collections (r = 0.877; P < 0.001), and 24-h urinary protein excretion and Uprot/UCr (r = 0.776; P < 0.001).

Triennial measurements of exogenous CCr demonstrated a significant decline of renal function in group 2 at 4 and 8 months of the study, reflecting the development of end-stage uremia in six dogs in this group during the first year of the study (Figure 1). In contrast, there were no significant differences in mean CCr between surviving dogs of group 1 and group 2 at 16, 20, and 24 months. Linear regression
Mineral Restriction in 15/16 Nx Dogs

**Figure 1.** Mean values during stage II for exogenous CCr in dogs fed a 0.44% P (group 1) or 1.50% P (group 2) mineral diet after 15/16 Nx. Number of surviving dogs at each time period is enclosed in parentheses for group 1 and brackets for group 2. Renal function was preserved at 4 and 8 months of stage II with dietary mineral restriction (*P < 0.05*).

**Figure 2.** Survival in dogs fed a 0.44% P (group 1) or 1.50% P (group 2) diet after 15/16 Nx. Survival was improved with dietary mineral restriction (*P < 0.01*).

Analysis was used to determine the rate of change of CCr over time for each dog as outlined above, after a method previously employed to analyze heterogeneous renal functional responses (5). The mean $r^2$ for all linear regression models was 0.71 ± 0.08. Although some dogs in both groups had decrements of renal function, reduction of dietary mineral intake was associated with a slowing of the rate of progression from 11.1 ± 3.6% decline in CCr/month in group 2 to a value of 2.6 ± 1.1% decline in CCr/month in group 1. As a consequence of this preservation of renal function, survival was improved by reduction of mineral intake (Figure 2), with a 75% survival rate at the end of the 24-month dietary trial (stage II) compared with a 33% survival rate at the comparable time in group II (*P < 0.01*). For each dog that was sacrificed during stage II, necropsy findings were consistent with uremia due to parenchymal renal...

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**Table 1.** Mean values for renal and systemic parameters during the 24-month dietary study (stage II) in partially Nx dogs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (kg)</td>
<td>12.9 ± 0.8</td>
<td>10.8 ± 0.6</td>
<td>11.1 ± 1.1</td>
</tr>
<tr>
<td>CCl (mg/dl)</td>
<td>741 ± 4.0</td>
<td>640 ± 3.0</td>
<td>610 ± 2.0</td>
</tr>
<tr>
<td>Plasma protein (g/dl)</td>
<td>6.1 ± 0.4</td>
<td>5.6 ± 0.5</td>
<td>5.8 ± 0.6</td>
</tr>
<tr>
<td>Plasma creatinine (mg/dl)</td>
<td>0.38 ± 0.04</td>
<td>0.36 ± 0.03</td>
<td>0.37 ± 0.04</td>
</tr>
<tr>
<td>Plasma cholesterol (mg/dl)</td>
<td>200 ± 10</td>
<td>180 ± 9</td>
<td>190 ± 11</td>
</tr>
<tr>
<td>Plasma triglycerides (mg/dl)</td>
<td>80 ± 4</td>
<td>70 ± 3</td>
<td>75 ± 5</td>
</tr>
<tr>
<td>Plasma calcium (mg/dl)</td>
<td>8.4 ± 0.4</td>
<td>8.1 ± 0.3</td>
<td>8.3 ± 0.5</td>
</tr>
<tr>
<td>Plasma phosphorus (mg/dl)</td>
<td>3.6 ± 0.2</td>
<td>3.4 ± 0.1</td>
<td>3.5 ± 0.2</td>
</tr>
</tbody>
</table>

*Values are mean ± se of the average value for each dog during stage II.*

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**Notes:**
- *P < 0.05 versus group 2.
- *P < 0.01 versus group 2.
- *P < 0.001 versus group 2.

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**References:**
failure and other nonrenal contributory factors were not identified.

Plasma PTH concentrations were similar in groups 1 and 2 at the end of stage I (Table 2), before the institution of the dietary study (stage II). Two months after the introduction of the high-phosphorus diet in group 2, there was a significantly greater elevation of plasma PTH concentrations in this group (243.1 ± 77.6 versus 106.5 ± 23.8 pg/mL; \( P < 0.05 \)). As renal function declined in group 2, progressively rising mean values for plasma PTH were noted (midpoint value of 280.5 ± 73.7 pg/mL and terminal value of 446.8 ± 119.4 pg/mL). Although there was a similar trend in plasma PTH concentrations noted in group 1 (midpoint value of 205.4 ± 31.8 pg/mL and terminal value of 406.5 ± 113.7 pg/mL), there was a significant \( (P < 0.05) \) enhancement of plasma PTH concentration in dogs on the higher phosphorus diet (group 2). Although values for plasma phosphate concentration were not different between groups at the end of stage I (Table 2), values at 4 months \( (8.1 ± 1.7 \text{ versus } 5.0 ± 0.5 \text{ mg/dL}) \), 8 months \( (6.4 ± 0.8 \text{ versus } 5.1 ± 0.4 \text{ mg/dL}) \), 12 months \( (8.3 ± 2.7 \text{ versus } 7.3 ± 2.4 \text{ mg/dL}) \), 16 months \( (5.5 ± 1.3 \text{ versus } 5.1 ± 0.4 \text{ mg/dL}) \), 20 months \( (6.5 ± 2.1 \text{ versus } 5.8 ± 0.6 \text{ mg/dL}) \), and 24 months \( (9.9 ± 4.6 \text{ versus } 9.7 ± 2.4 \text{ mg/dL}) \) of the dietary study demonstrated a consistent trend for values in group 2 to exceed those for group 1 and this effect of dietary phosphorus on plasma phosphate concentration was significant \( (P < 0.05) \).

The mean value of all measurements made during stage II was determined for each dog (Table 3). Renal function, as assessed by CCr and plasma creatinine concentrations, was improved by dietary mineral restriction in group 1. The average of all trimester values in group 2 for FEP \( (33.1 ± 3.3\%) \) and monthly values for plasma phosphate concentration \( (7.98 ± 0.67) \) were significantly greater than the corresponding values for group 1 \( (19.4 ± 2.3\%; P < 0.05; \text{ and } 6.24 ± 0.45 \text{ mg/dL}; P < 0.05) \). Mean values for total CO\(_2\) were significantly lower in group 2 and the mean value for plasma triglyceride concentration was higher in dogs fed the higher mineral content diet. The Uprot/Ucr ratio was elevated in group 1. The final Uprot/Ucr in group 1 averaged 3.12 ± 0.44, which was significantly greater \( (P < 0.05) \) than the initial value of 0.86 ± 0.18 for the same group. Although the same trend was noted in group 2, with a final mean value of Uprot/Ucr of 1.45 ± 0.44 versus the initial value of 0.69 ± 0.14, this difference was not statistically significant and the final value for group 2 was less \( (P < 0.05) \) than the corresponding final value for group 1. The final Uprot/Ucr was positively correlated with the time of sacrifice \( (r = 0.491, N = 24, P < 0.05) \). There were no differences between groups in mean values for hematocrit, MAP, or plasma concentrations of calcium, protein, or cholesterol. Body weight was not different between groups and remained stable throughout stage II in both groups.

In group 2, fatality occurred sooner \( (6.1 ± 1.4 \text{ versus } 13.0 ± 1.0 \text{ months in group } 1; P < 0.05) \) and more frequently \( (66.7 \text{ versus } 25\% \text{ fatality rate in group } 1) \). Fatality was not correlated with degree of glomerular lesions, proteinuria, or glomerular hypertrophy. However, in each group progression to end-stage uremia was associated with decrements of CCr, increased renal mineralization, and an elevated tubulointerstitial index (Table 4). Renal mineralization was most prominent in cortical tubular epithelia and basement membranes in both groups. Renal calcium accumulation was accompanied by magnesium and phosphorus accumulation. Phosphorus content in outer cortical tissue from uremic dogs that were sacrificed during stage II \( (N = 11) \) averaged 9,856 ± 1,638 ppm and exceeded \( (P < 0.05) \) the corresponding value measured in dogs that survived stage II \( (5,480 ± 918; N = 13) \); whereas phosphorus content of the inner cortex/medulla was similar in kidneys from stage II fatalities \( (6,893 ± 964 \text{ ppm}) \) and from surviving animals \( (4,919 ± 664 \text{ ppm}) \). The mean magnesium content in the outer cortex of uremic dogs \( (374 ± 69 \text{ ppm}) \) was greater \( (P < 0.05) \) than that of surviving dogs \( (209 ± 43 \text{ ppm}) \). A similar trend for magnesium accumulation was noted in the inner cortex/medulla of uremic dogs \( (278 ± 48 \text{ versus } 188.7 ± 30 \text{ ppm}; NS) \).

 Although fatality was due to loss of renal function in dogs of each group, many of the surviving dogs also had decrements in CCr. To determine which factors were associated with declining renal function, linear regression relationships between the rate of decline of CCr during stage II (determined by linear regression analysis of CCr versus time for each dog) and MAP, the Uprot/Ucr, plasma phosphate, calcium, PTH, triglyceride, and cholesterol concentrations, FEP, glomerular volume, glomerular and tubulointerstitial histologic indices, and renal calcium concentrations were determined. Because the distribution of values for renal calcium concentrations were skewed by a few extremely high values, the log\(_{10}\) of tissue calcium concentrations were employed to provide a normal distribution of values. For this analysis, MAP, FEP, the Uprot/Ucr, and plasma phosphate, triglyceride, and cholesterol values were taken as the average of all observations obtained during stage II for each dog. Those variables with a significant \( (P < 0.05) \) linear relationship with the rate of decline of CCr in group 1 were log\(_{10}\) of inner cortical/medullary calcium concentration, log\(_{10}\) of outer cortical concentration, tubulointerstitial index, plasma concentrations of triglycerides and cholesterol, the Uprot/Ucr, and glomerular index (Table 5). For group 2, variables with a significant \( (P < 0.05) \) relationship with the rate of decline of CCr were plasma PTH, tubulointerstitial index, plasma phos-
TABLE 4. Final values for renal functional and morphologic parameters in partially Nx dogs that were sacrificed when they developed terminal uremia during stage II (fatalities) and those that remained in the study through stage II (survivors) grouped according to diet

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Dogs (N = 12)</th>
<th>Time of Sacrifice (months)</th>
<th>Clcr (mL/min/kg)</th>
<th>CCr (mL/min/kg)</th>
<th>MAP (mm Hg)</th>
<th>Histologic Indices</th>
<th>Renal Structure Size</th>
<th>Renal Calcium Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tubulointerstitial Index</td>
<td>Glomerular Index</td>
<td>Kidney WT (g)</td>
</tr>
<tr>
<td>Survivors</td>
<td>9</td>
<td>24.0</td>
<td>0.54±0.06</td>
<td>0.65±0.05</td>
<td>117.1±0.01</td>
<td>1.00±0.02</td>
<td>1.41±0.02</td>
<td>21.6±3.3</td>
</tr>
<tr>
<td>Fatalities</td>
<td>3</td>
<td>13.0</td>
<td>0.118±0.001</td>
<td>0.114±0.000</td>
<td>145.0±0.03</td>
<td>2.03±0.07</td>
<td>1.77±0.10</td>
<td>21.1±3.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.027±0.001</td>
<td>0.036±0.000</td>
<td>1.39±0.02</td>
<td>-0.02±0.02</td>
<td>-0.22±0.02</td>
<td>7.2±0.027</td>
</tr>
</tbody>
</table>

Group 2 (N = 12)

| Survivors | 4             | 24.0                       | 0.766±0.005      | 0.786±0.001     | 117.0±0.05 | 0.54±0.02         | 1.30±0.02         | 24.5±3.4     | 5.351±0.036 | 7.35±0.014 | 425±0.017  | 541±0.022 |
| Fatalities | 8             | 6.1                        | 0.134±0.001      | 0.134±0.001     | 128.5±0.08 | 2.00±0.05         | 0.56±0.08         | 18.6±0.06   | 3.066±0.014 | 2.14±0.007 | 3.752±0.038 | 2.180±0.038 |
|           |               |                            | 0.038±0.001      | 0.037±0.001     | 0.18±0.02  | -0.18±0.02        | -0.22±0.02        | 5.5±0.022    | 1.88±0.022 | 0.22±0.008 | 1.57±0.030 | 0.55±0.058 |

* Group 1, 0.44% P diet; group 2, 1.50% P diet. Values are mean ± SE. For fatalities, values represent the terminal measurements before sacrifice. For survivors, final values are those obtained at the end of stage II. Values within the same column with no matching superscripts are significantly different (P < 0.05).

TABLE 5. Regression relationships between the rate of decline of CCr and other variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log Uprot/UCr</td>
<td>0.003</td>
<td>0.007</td>
</tr>
<tr>
<td>Glomerular Index</td>
<td>0.478</td>
<td>0.478</td>
</tr>
<tr>
<td>Tubulointerstitial Index</td>
<td>0.478</td>
<td>0.478</td>
</tr>
<tr>
<td>Plasma phosphatase</td>
<td>0.478</td>
<td>0.478</td>
</tr>
<tr>
<td>Plasma alkaline phosphatase</td>
<td>0.478</td>
<td>0.478</td>
</tr>
<tr>
<td>Log Ca²⁺</td>
<td>0.478</td>
<td>0.478</td>
</tr>
<tr>
<td>Log inner cortical/medullary</td>
<td>0.478</td>
<td>0.478</td>
</tr>
</tbody>
</table>

For the analyses, mean values for all stage I measurements were used. Only significant relationships are reported.
The protective role of phosphorus restriction in renal failure is not clear because some studies of phosphorus restriction did not control food intake (3,15) and others have failed to identify a protective role for phosphorus restriction (24). Most studies of the role of phosphorus in the progressive nephropathy of rats have used diets with marked variations in the Ca:P ratio. Because dietary Ca:P imbalance may lead to nephrocalcinosis and/or nutritional hyperparathyroidism irrespective of the level of phosphorus intake (21-23), dietary Ca:P balance was purposely maintained constant in the diets of the study presented here. Since the addition of calcium to the diet will most likely mitigate the untoward effects of dietary phosphorus intake (21-24), the chosen method of dietary mineral variation is a more rigorous test of the role of phosphorus in the progression of renal failure. Results do demonstrate that increased availability of dietary phosphorus is associated with accelerated progression of renal failure in dogs.

The phosphorus content of the diets (0.44% in diet 1, 1.50% in diet 2) compares with an average reported mean phosphorus content of 1.30% in commercially available preparations (36). The mean daily phosphorus intake in group 1 (71 mg of phosphorus/kg body wt) was below the recognized minimum phosphorus requirement for adult maintenance in dogs of 89 mg of phosphorus/kg body wt (37). Nevertheless, our balanced mineral restriction did not result in hyperphosphatemia or hypophosphaturia. Although more severe in group 2, elevations of FEP and plasma concentrations of phosphate and PTH were observed in both groups of partially Nx dogs throughout the study. Phosphorus imbalance and hyperparathyroidism may have contributed to progressive renal failure and fatality in both groups of dogs.

Decrement of renal function were more closely related to nephrocalcinosis and severity of tubulointerstitial lesions than to glomerular pathology. Previous investigators have implicated nephrocalcinosis and progressive tubulointerstitial disease in models of phosphorus toxicity that use rats (3,14,15,18,38,39). In our dogs, progression and fatality were associated with interstitial fibrosis, tubular atrophy and dilation, and mineralization of cortical basement membranes, tubular epithelia, and vascular and tubular lumina. The association of progression with tubulointerstitial lesions and nephrocalcinosis does not necessarily establish a causal relationship. However, other studies have supported a causal role for nephrocalcinosis. Prevention of nephrocalcinosis by phosphorus depletion (3,14,15) or by administration of agents to block nephrocalcinosis (16,17,39) protects renal function in rats.

Increased calcium deposition in kidneys of female dogs was apparent by mineral analysis and morphologic studies. This gender-dependent calcium depo-

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**DISCUSSION**

A pattern of declining renal function developed in 10 of 12 dogs maintained on diet 2 during the 24-month feeding trial after 15/16 Nx. Although the temporal pattern of renal function was heterogeneous during stage II, declining renal function led to the development of end-stage renal failure in 8 of 12 dogs in group 2. Reduction of dietary mineral intake slowed the rate of progression and improved survival, independent of dietary intake of protein, energy, fat, sodium, potassium, or magnesium. This phosphate-dependent progression was most evident during the first year of stage II, and further differences between groups in mean renal function were not apparent beyond this time. Progression to end-stage renal failure was associated with renal mineralization and the development of tubulointerstitial lesions in each group. These changes were more severe and occurred earlier in female dogs and were accelerated by higher dietary mineral intake.

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**Figure 3.** Rate of decline of exogenous CCr (upper panel) and mean survival time (lower panel) in male and female dogs fed a 0.44% P (group 1) or 1.50% P (group 2) diet after 15/16 Nx. The dietary effect of phosphorus was most pronounced in female dogs. Bars with no shared superscripts are significantly different (P < 0.05).

Plasma concentrations of phosphate, calcium, electrolytes, cholesterol, or triglycerides.
situation was enhanced in both diet groups, although most prominent in group 2. It was associated with accelerated decline of renal function and an increased fatality rate for female relative to male dogs. An estrogen-dependent component to nephrocalcinosis has been reported in rats (26).

A role for PTH in experimental renal injury has been proposed by others (14,18,19) and progression of renal failure in human beings is related to uncontrolled hyperparathyroidism (40) and phosphorus imbalance (41). Decline of GFR was more rapid in dogs with the higher mineral intake and was associated with elevations of plasma PTH, FEPh, and plasma phosphate concentration. Metabolic acidosis (and consequent enhancement of renal ammoniagenesis) may have participated in the development of tubular-interstitial lesions, an effect suggested by results of studies of rats with renal disease (4). The development of acidosis (decreased plasma total CO2 content) and tubular-interstitial lesions was evident in dogs with declining Ccr, and a role for enhanced renal ammoniagenesis cannot be excluded.

Whereas some dogs develop elevations of MAP, glomerulosclerosis, proteinuria, and progressive decrements of renal function after partial Nx, results of this and previous (9–13,42) studies demonstrate that most do not. It is apparent that a modest reduction of renal mass does not lead to progressive glomerulosclerosis and GFR decline in female beagles (9,10). Unlike rats, a severe degree of Nx (7/8 or greater) is needed in dogs and, even then, GFR will not decline in all dogs (13; this study). Many studies have suggested a role for glomerular capillary hypertension (1,2,7), glomerular enlargement (43), and glomerulosclerosis in the progressive nephropathy of rats, and some studies of renal failure in rats have demonstrated that phosphorus restriction is protective of glomerular morphology (14,15) and/or reduces proteinuria (14). Glomerular capillary hypertension and glomerular enlargement are also present in remnant nephrons of dogs after partial Nx (44; this study). Although glomerular lesions, increased glomerular volume, and modest proteinuria were evident in surviving animals, our dogs did not develop the severe proteinuria and abnormal glomerular morphology associated with progressive renal dysfunction in rats. These findings suggest that glomerulosclerosis is a slow process and not an important factor of progressive renal dysfunction in dogs.

In brief, results of the study presented here establish that a progressive decline of renal function was uncovered in dogs by marked partial Nx. In this setting, the rates of progression and fatality were related to hyperphosphatemia and hyperparathyroidism. Renal morphologic injury and functional decline were associated with renal mineralization and tubular-interstitial lesions rather than glomerular pathol-

ogy and proteinuria. These events developed more readily in female than in male dogs and were accelerated by higher dietary mineral intake.

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REFERENCES


